Technical Papers

Relationships of Fungi, Mites, and the Potato-rot Nematode[1]

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Frequent examinations of potato tubers infested with potato-rot nematode, *Ditylenchus destructor* Thorne, 1945 have disclosed a variety of conditions within the invaded areas, and in 1947 the senior author (*1*), reporting on experimental transfers, found that the progress of injury may vary widely between tubers even though they are all of the same variety from the same source and all inoculated on the same date with nematodes belonging to a common original population. Sometimes the destruction of potato tissue is rapid and may progress with little or no interruption until the greater part of the tuber is affected. In other cases, no appreciable injury to the tuber occurs even though live nematodes may be found near the point of inoculation 6 or 8 wk after their introduction. Several intermediate conditions have been noted. The possible relation of associated organisms, such as bacteria, fungi, mites, and other nematodes has been given attention. Wet rots (bacterial) appear to produce conditions unfavorable to the nematode. As a general rule, complete destruction of the potato tuber is not brought about by the nematodes, as a point seems to be reached where secondary, or associated, organisms complete the destruction. When this has occurred, it is difficult and usually impossible to locate any surviving nematodes; and, although it has been assumed that under natural conditions they return to the soil when the tuber breaks down, it now seems likely that at least the greater part of the population in a potato crop succumbs.

During many detailed examinations of infested potatoes it was observed that sometimes, where a large part of the tuber had been destroyed, there were very few nematodes in the bordering healthy potato tissue, and yet there were large populations in the areas that had been broken down. In such cases, fungus growth was abundant in the latter area. As it appeared likely that the nematodes were feeding on the fungus, both nematodes and fungi were transferred to Petri dishes containing potato dextrose agar, and the nematodes were propagated in this way. These results were confirmed later by repeated successful inoculations of fungus plates with the potato-rot nematode.

Nematodes in these fungus plates tend to assemble in and around the fungus colonies, and they have been frequently observed feeding on the mycelium. It is now evident that live potato tissue is not essential for the propagation of these nematodes, and transfers to potato agar without the fungus colonies were invariably unsuccessful.

In 1951 Seinhorst and Bels (*2*) reported *Ditylenchus destructor* as attacking mushrooms, and Cairns (*3*) has more recently reported *Ditylenchus* sp. feeding on this host in the United States. The fungus cultures that the potato-rot nematode will feed on may include many species. Host fungi already identified at the Ottawa laboratory include three species of *Fusarium,* a species of *Mucor,* possibly *M. hiemalis* Wehmer, and *Trichoderma viride* Pers. ex. Fr.[2] On observing these nematodes with their stylets inserted in a fine strand of mycelium considerably less than their own diameter, one wonders how much nourishment can be obtained from each puncture. However, with fungi having nonseptate mycelium, the efficiency of the operation becomes more understandable.

Mites have been frequently found in association with nematode-infested tubers. It has also been observed that when the mite populations were large the nematode populations declined sharply. In the culturing of these nematodes on fungus plates, the mites fed on the fungi, and they can injure a fungus colony very quickly. If the fungus is largely consumed the nematodes disappear, and it is now indicated that this is what may take place frequently in potato tubers.

In an earlier paper (*2*) the "feeding front" of these nematodes within potato tubers was referred to. It is behind this feeding front that a "fungus garden" is built up in the tuber, and one may speculate that the main activity of the nematodes is in building up this garden rather than in direct feeding on live potato tissue. When this fungus garden has been well established, most of the nematodes are found in this area. There is at least some indication that fungus colonies may be necessary for the most successful development of these nematodes in the potato. If mites gain entrance to a nematode-infested tuber the fungus garden is often destroyed and the nematodes disappear. A species of mite that attacks these fungi is *Tyrophagus putrescentiae* (Schr.); another species, *Histiostoma feroniarum* (Dief.), has been identified.[3]

To solve the species problem that may be involved in these potato-rot nematode populations, many attempts have been made to build up pure colonies of the nematodes through single-female transfers to potato tubers. That this could be done was first demonstrated here in 1947. Greater attention has been recently given to this method. A number of these single-female colonies have been established but only after a very large number of attempted transfers. With the development of our technic for rearing these nematodes on fungus cultures, the single-female colonies were transferred to fungus cultures, with the idea that

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by this means they could be more rapidly built up and maintained. In most cases these transfers have been very successful, only a few of the colonies have succumbed. The failures may have been due to the normal hazards accompanying transfers, but the possibility is recognized that a species complex may exist in the potato populations. It is possible that intimate relationships between plant-parasitic nematodes and fungi may prove to be more common and important than has been hitherto suspected.

References

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The Separation of Neuroblasts and Other Cells from Grasshopper Embryos[1]

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In the course of our studies of the effects of x-rays on the metabolism of grasshopper embryos (*Chortophaga viridifasciata* DeGeer), it was desirable to have suspensions of free cells in as near normal morphological and physiological condition as possible. Adequate suspensions have been obtained through enzymatic digestion of the intercellular substance. Neuroblasts as well as other types of embryonic cells from these suspensions undergo mitosis and maintain active respiration for several hours after separation. The method developed, somewhat similar to the method of separation of chick embryo cell suspensions described by Moscona (*1*), utilizes a digestive mixture of trypsin and hyaluronidase in a calcium-free medium at pH 7.4. Trypsin was essential for adequate solution of the intercellular substance, whereas the development of gel in the medium was lessened considerably by the addition of hyaluronidase. It is well known that calcium-free media decrease the adhesiveness of embryonic cells (*2, 3*) and cells of mature invertebrate and vertebrate tissues (*4–7*).

Routinely, 150 embryos (average total dry weight, 5.25 mg), selected morphologically at a stage equivalent to that of 14 days development at 26° C, were removed from the eggs under aseptic conditions in an isotonic salt medium containing calcium. After rinsing twice in a calcium-free medium to remove residual calcium, yolk, and egg fluid, the embryos are placed in 5 ml of digestive mixture for 1 hr at 38° C. The digestion mixture consisted of 7.5 mg each of recrystallized trypsin (Worthington) and bovine testis hyaluronidase (Armour or Mann) in 10 ml of calcium-free solution. Agitation during the period of digestion was avoided as this resulted in very viscous solutions, or gel formation, and clumping of the embryos. At the end of the hour 0.5 ml of protein solution (5 mg of Armour bovine albumin/0.5 ml calcium-free medium) was added to the digest suspension. Holter and Zeuthen (*8*) used egg white to decrease the adhesiveness of ascidian eggs to glass or to one another, and in our experience the albumin aided in overcoming stickiness among separated cells. At this stage the general form of the embryos was still distinguishable. Individual cells were separated by forcing embryos and solution slowly into and out of a small pipet with a rather large bore. Separation must be done carefully and gently for mechanical damage results in "bleb" formation at the cell surface, and few or no dividing cells can subsequently be observed in the suspension. When the solution is quite milky and all large particles have been broken up, it is transferred to a centrifuge tube with 5 ml of culture-medium containing calcium. After centrifugation at 158 rpm on a clinical centrifuge for 15 min (distance from axis to bottom of tube, 21 cm), the residue was resuspended in 0.6 ml of fresh medium. Cell separation was fairly complete, and no evidence of reaggregation of separated cells has been obtained. Hemocytometer counts of a typical suspension gave 2,000,000 cells, or about 13,300 cells/embryo, a figure which is undoubtedly low because of the unavoidable loss of cells during separation.

In Fig. 1 is shown the endogenous oxygen consumption of a cell suspension containing 1,732,000 cells measured at 38° C, in an atmosphere of oxygen. Respiration decreases after 3 hr, possibly due to lack of substrate. The average rate of oxygen consumption of the suspension shown in Fig. 1 was 12.7 mm^3 of oxygen/hr at 38° C. In Warburg determinations of the respiration of intact embryos, we have obtained an average value of 31.5 mm^3 of oxygen/hr for 150 embryos. The difference between these two values, 31.5 and 12.7, may be a measure of the yield of embryonic cells in the suspension, but with the evidence at hand

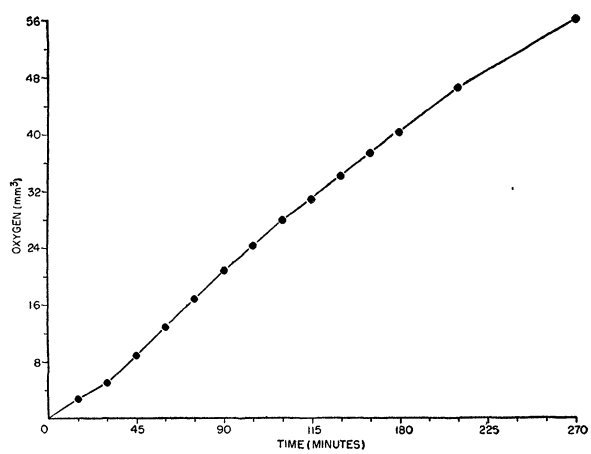

FIG. 1. The endogenous respiration of a grasshopper embryonic cell suspension in isotonic salt solution.

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